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Abstract: Glioblastoma is the most aggressive primary tumor of the central nervous system with a medium overall survival of 7-15months after diagnosis. Since tumor cells penetrate the surrounding brain tissue, complete surgical resection is impossible and tumor recurrence is almost a certainty. New treatment modalities are therefore needed, and these should be able to trace, identify, and kill dispersed tumor cells with great accuracy. Immunological approaches in principle meet these needs. Unfortunately, due to profound tumor-associated mechanisms of immunosuppression and -evasion, immunotherapeutic strategies like peptide vaccination have so far not been translated into clinical success. If future, peptide-based vaccination approaches shall be successful in glioblastoma therapy, multiple questions need to be solved including identification of suitable antigens, route and mode of vaccination, preparation of the tumor-bearing "host" and antagonizing, as much as this is possible, glioblastoma-associated mechanisms of immune evasion and poor vaccination response. In this review we will address the immunological challenges of glioblastoma and discuss key aspects that have rendered successful immunotherapy difficult in the past.

DOI: https://doi.org/10.1016/j.ctrv.2013.08.008

Originally published at:
Mohme, Malte; Neidert, Marian C; Regli, Luca; Weller, Michael; Martin, Roland (2014). Immunological challenges for peptide-based immunotherapy in glioblastoma. Cancer Treatment Reviews, 40:248-258.
DOI: https://doi.org/10.1016/j.ctrv.2013.08.008

Immunological Challenges for Peptide-based Immunotherapy in Glioblastoma

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Running title: Immunological Challenges in Glioblastoma Vaccination

ABSTRACT

Glioblastoma is the most aggressive primary tumor of the central nervous system with a medium overall survival of 7-15 months after diagnosis. Since tumor cells penetrate the surrounding brain tissue, complete surgical resection is impossible and tumor recurrence is almost a certainty. New treatment modalities are therefore needed, and these should be able to trace, identify, and kill dispersed tumor cells with great accuracy. Immunological approaches in principle meet these needs. Unfortunately, due to profound tumor-associated mechanisms of immunosuppression and -evasion, immunotherapeutic strategies like peptide vaccination have so far not been translated into clinical success. If future, peptide-based vaccination approaches shall be successful in glioblastoma therapy, multiple questions need to be solved including identification of suitable antigens, route and mode of vaccination, preparation of the tumor-bearing "host" and antagonizing, as much as this is possible, glioblastoma-associated mechanisms of immune evasion and poor vaccination response. In this review we will address the immunological challenges of glioblastoma and discuss key aspects that have rendered successful immunotherapy difficult in the past.

KEY WORDS:

Glioblastoma; Glioma; Immunotherapy; Peptide-vaccination; Tumor immunology; Anti-tumor response

INTRODUCTION

Glioblastoma is the most common primary malignancy of the central nervous system and represents almost 50% of all primary intracranial neoplasias.[1] Because of the early infiltration of surrounding tissue, the high recurrence rate with fast progression, and the inability to completely eliminate the tumor glioblastoma evades successful treatment so far. Despite substantial advances and treatment refinements during the last decades, conventional therapies like neurosurgical resection and multimodal radio- and chemotherapy have limited effects on disease progression, recurrence rate or clinical outcome. Consequently, patients with newly diagnosed glioblastoma experience a median overall survival of only 7-15 months.[2,3] Despite extensive research, the prognosis for these patients has only improved by 3-6 months over the past decades.[4]

Although the clinical outcome is influenced by individual factors like MGMT-promoter-methylation,[5] isocitrate dehydrogenase (IDH) mutations, age and Karnofsky performance

score (KPS) at initial diagnosis,[6] as well as the location, configuration and surgical accessibility of the tumor, the dominating pathological feature of glioblastoma remains its high recurrence rate. While metastases to other organs are rare, and less than 10% of malignant gliomas reoccur distant to the original site, local recurrence is almost certain.[7] A major factor responsible for the high recurrence rate is the ability of malignant cells to migrate and penetrate deeply into the surrounding parenchyma. Using white matter fiber tracts as well as feeding blood vessels as guiding pathways, tumor cells can spread and infiltrate anatomical structures adjacent to the primary tumor, so that dispersed tumor cells can be found centimeters away.[8,9] Since the brain does not allow expanded surgical en-bloc resection, neurosurgical treatment is restricted to reducing tumor burden. Therefore, while neurosurgical intervention remains one of the most important treatment approaches and the introduction of microscope-guided surgery improved the extent of surgical resection, complete surgical tumor removal remains impossible.[10]

Other treatment modalities such as radio- or chemotherapy using alkylating agents like temozolomide have increased the overall life expectancy, but their effects are limited. One reason for the intractability of glioblastoma is the transforming nature and dynamic molecular phenotype of glioblastoma, which includes multiple mechanisms to resist drug- and radiation-induced anti-tumor activity.[11,12] In addition, malignant gliomas are characterized by their heterogeneity, which is promoted by tumor-initiating cells that drive a constantly mutating cancer cell population.[13] The transforming nature of a heterogeneous tumor further facilitates the generation of defense mechanisms against radio- and chemotherapy. Although both treatment modalities prolong overall survival, they lack specificity and are accompanied by substantial side effects. New effective and more specific treatment modalities are therefore urgently needed.

Key Players in Immunotherapy

The immune system is not only engaged in defending the body from foreign pathogens, but it is also involved in eliminating cells that underwent malignant transformation in a process called “immune surveillance”.[14] Processes of malignant transformation are driven by genetic instability including changes in genes that are involved in cell cycle control, migration, angiogenesis, apoptosis, and also mutations of genes encoding for “normal” proteins not directly involved in tumor biology.[15] The immune system is able to recognize transformed cells and in analogy to vaccinations for infections, immune responses against tumor tissue can in principle be enhanced in an active or a passive fashion.[16] In contrast to

prophylactic vaccinations against infections, cancer immunotherapy aims at eradicating established diseases, i.e. is analogous to therapeutic vaccination.

The basic principle of cancer immunotherapeutic approaches is to evoke a tumor-specific cellular immune response resulting in the selective elimination of cancer cells. The central effector population for targeted cancer cell lysis is comprised of CD8+ T cells, also called cytotoxic T lymphocytes (CTL).[17] CD8+ T cells can identify antigenic peptides, which are presented by human leukocyte antigen (HLA) class I molecules on the surface of cancer cells with their antigen-specific T cell receptor (TCR).[18] The interaction of the TCR with the presented tumor antigen, together with costimulatory molecules like B7-1/2 will result in the targeted release of CTL effector molecules like perforin and granzyme, which induce apoptosis, as well as cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α/β (TNF-α/β). The potent CTL response is supported by a complex interaction of other immune cells, responsible for priming and amplification of the anti-tumor effect. An important part of the priming/activating immune cells are professional antigen-presenting cells (APCs), mainly represented by dendritic cells (DCs) which can take up tumor-associated proteins and/or peptides and, after intracellular processing, present them via HLA class I and -II molecules on their surface.[19] Although there is some cross-talk between these two processing and presentation pathways, presentation via HLA class I will mainly prime CD8+ CTL, while peptide binding to HLA class II will induce a CD4+ T_{helper} cell response.[20] Type 1 helper cells specific for tumor antigens are able to amplify CTL proliferation and enhance their anti-tumor effect. By creating a local proinflammatory environment, for example, by secreting cytokines like IL-2 or IFN-γ among others T_{helper} cells promote the local reactivation of CTL by APC. This means, in order to mount an efficient tumor-specific immune response, both CTL as well as T_{helper} cell activation against tumor antigens that are presented in the context of HLA-class I and -class respectively are required (Figure 1).

As we would like to focus on immunological challenges that are relevant to improve immunotherapeutic protocols in a clinical setting, we will not introduce all molecules and cell populations, for example NK- and NK-T-cells, that are additionally involved in the complex interaction between immune- and cancer cells, but focus on the adaptive T cell-mediated and tumor specific immune responses.

Peptide-based immunotherapeutic approaches

Tumor vaccination protocols can either be based on vaccination with a peptide or protein, ideally one that is specific and relevant for the respective tumor. Different protocols

using tumor-specific peptide antigens have been established. Crude peptide digests of the autologous tumor can be used for vaccination, but in most cases synthetically manufactured peptides are injected subcutaneously or intranodally to prime the host immune system and to expand existing tumor-specific CTL.[21] Theoretically, if the immune response is strong enough and sufficient numbers of tumor-specific CD8+ T cells expand, cell-mediated lysis of the tumor cells could lead to tumor regression,[22] and a cure is at least a possibility. Another immunotherapeutic approach is the adoptive transfer of autologous or genetically engineered tumor-specific T cell populations. Although adoptive T cell transfer harbors great potential, we will focus here on the more clinically feasible peptide-based immunotherapeutic approaches.

Another approach of inducing a tumor-specific immune reaction is dendritic cell vaccination. Ralph Steinman`s discovery of DCs and their potent antigen presenting function provided the rationale for DC vaccination protocols. Compared to peptide vaccination, vaccination with peptide-pulsed DCs is considerably more labor-intensive, requires a GMP laboratory, and therefore poses both technical and financial hurdles. It involves the isolation of large numbers of peripheral blood mononuclear cells (PBMCs) from the patient using leukapheresis. Out of the heterogeneous cell mixture of PBMCs, CD34+ or monocytic cells /language></record></Cite></EndNote>¶ 110⊥ ⊥ Modifications like myristilation, amino acid substitutionfter exposure to whole tumor cells, tumor cell lysates, tumor RNA or specifically selected peptide mixtures, the pulsed dendritic cells are readministered to the patient. Due to the excellent antigen presentation and density of costimulatory molecules, mature DCs in consequence are able to effectively stimulate the expansion of tumor-specific T cells.[23] As for peptide vaccination, the selection of the appropriate antigen for the loading of DCs is of major importance. Approaches that use unspecific tumor extracts carry the risk of severe autoimmune collateral damage.[24]

Limits of current immunotherapeutic approaches

A substantial number of clinical vaccination trials for malignant gliomas have been conducted. While tumor immunotherapeutic approaches in animals have led to significant tumor reduction and produced long-term tumor immunity, anti-tumor efficacy in human trials has so far been disappointing throughout all cancer entities including glioblastoma. Despite many phase I- and an increasing number of phase II trials, only one peptide vaccine for hormone-refractory prostate cancer was approved by the FDA.[25] A metaanalysis by Rosenberg et al. summarized the poor results of peptide vaccination trials that were

performed until 2006. Using objective criteria of tumor response, of 440 patients only 12 (2.6%) responded to vaccination treatment.[26]

The *in vivo* response rate has been particularly low in high-grade gliomas and resistance towards an immune-mediated tumor regression is a hallmark of glioblastoma.[27] It appears that not only the initiation, but also the execution of tumor-directed effector functions poses considerable obstacles in the treatment of glioblastoma. To achieve clinical success, several factors like efficient T cell activation, selection of the best antigens and peptides, immunogenic presentation as well as ways to overcome glioblastoma-associated immune evasion mechanisms or immunosuppression, have to be considered. Considering the sobering results of clinical trials, but also the at least theoretically promising advances in tumor immunology we decided to review and critically discuss immunological aspects of vaccination strategies to treat glioblastoma.

Promising data from clinical studies

It has been shown for other malignancies that peptide-based immunotherapy is able to mount a safe and effective immune response. In a randomized phase 3 trial enrolling 185 patients with advanced melanoma, the vaccine, consisting of the HLA*A02:01-restricted peptide gp100:209-217(210M) plus incomplete Freund`s adjuvant, was administered followed by interleukin-2 and compared to interleukin-2 alone. The vaccine group had a significantly higher response rate, as well as longer progression-free survival than the control group, which received the cytokine alone.[28] A therapeutic vaccine for HLA-A*02+ renal cell cancer patients consisting of multiple tumor-associated peptides was safe and already showed improved disease control in a phase 1 trial. Immunomodulation using single-dose cyclophosphamide with the aim of purging regulatory T cells that prevent efficient tumor-specific immune activation has been linked to prolonged survival among those patients, who mounted a specific immune response to the vaccine.[29]

As the first trial advancing to phase III testing, peptide-vaccination targeting the epidermal growth factor receptor (EGFR) mutation III (vIII) is currently one of the most prominent examples for immunotherapy for glioblastoma.[30,31] The preceding EGFRvIII trial results can offer important insights into *in vivo* dynamics of a peptide vaccine, which we would like to discuss briefly from an immunological perspective. In general, the EGFR is a tyrosine kinase overexpressed in up to 50% of tumors.[32] The EGFRvIII is a mutated form of this receptor, harboring a constant deletion within the extracellular domain. The peptide used in the EGFRvIII trials spans this mutated EGFR region, thereby displaying a tumor-specific

neoantigen for T cell-mediated immunotherapy that is not expressed in normal tissue. Although this mutation is present in up to 20% of high-grade gliomas, the high tumor heterogeneity results in a large proportion of cells (24-63%), which do not express this antigen.[30,33]

A phase II trial, named ACTIVATE, applied the classical peptide vaccination protocol. The treatment regimen consisted of surgical tumor resection (gross total resection > 95%) followed by radiotherapy combined with temozolomide treatment. After completion of chemotherapy the patients had to be progression free for 4 weeks before being vaccinated with the peptide. The 14-amino-acid long, EGFRvIII peptide was administered subcutaneously every two weeks together with an immune booster (GM-CSF). Patients were monitored every two months with MRI. If radiographic progression was detected, therapy was discontinued. Results demonstrated prolonged progression free survival and mean overall survival of vaccinated patients compared to a historically matched cohort.[34,35] Clinical efficacy of the EGFRvIII peptide vaccination will be further assessed in an international multi-center randomized double blind placebo controlled trial called ACT IV.

So far, the EGFRvIII trials generated two immunologically interesting results relevant for future glioma vaccinations. First, patients, in whom an EGFRvIII-specific antibody response was detected, had a significantly prolonged overall survival raising the interesting point that not only T cell-mediated, but also humoral, i.e. antibody-mediated, tumor-specific immune responses can be mounted. This implies a correlation of successful induction of immunity and the consequent translation into clinical success. Second, 82% of all recurring tumors demonstrated loss of the EGFRvIII mutation,[35] indicating a successful induction of an immune-mediated tumor cell lysis, however, at the same time evasion of the tumor. The turnover, high prevalence of mutations and the great heterogeneity of glioblastoma seem to easily overcome vaccination with a single antigen. Although, the selection of EGFRvIII negative tumor cells can possibly promote a more indolent tumor expansion, a plethora of growth-enhancing mutations ensures continuous cell growth, which then also might lead to an exceedingly aggressive phenotype.

With a great variety of clinical trials using immunotherapy for cancer, additional interesting data can be expected in the near future. Table 1 summarizes ten ongoing clinical trials using peptide-based vaccination to apply immunotherapy to glioblastoma.

IMMUNOLOGICAL CHALLENGES OF GLIOBLASTOMA

Immunotherapy can be successful because not only *in vitro* but also *in vivo* studies have shown that it is possible to induce a tumor-specific immune response. Glioblastoma patients exhibit significant *in vivo* frequencies of tumor-specific T cells without prior stimulation via a vaccinating agent.[36] Experiments using murine models demonstrated that long-term immunity and effective tumor rejection can be stimulated by vaccination.[37] Additional data dissecting human glioblastoma tissue phenotypes further discovered a correlation between the magnitude of CD8+ T cell infiltration and a prolonged overall survival.[38,39] Such data provides evidence of the immune system`s ability to limit tumor progression. Unfortunately, until today clinical trials have not been able to translate these findings into clinical success. Since increasing evidence suggests the presence of a complex tumor-host interaction, we will review the immunological aspects of glioblastoma pathophysiology in the following sections.

Tumor evasion and immunosuppression

Malignant tumors are often depicted as a homogeneous cell population, which is characterized by uncontrolled cell growth. This view has changed over the past decades.[40] Tumors, and especially malignant gliomas, have been shown to interfere not only with the surrounding tissue, e.g. activating angiogenesis, but also to interact with the immune system.[27] Although the brain is conventionally considered an immunoprivileged organ, immune cells constantly cross the blood brain barrier, albeit to a lesser extent compared to other organs like skin or gut. Since the immune system is able to identify malignant cells in the early stages of tumor formation in a process called cancer immunosurveillance,[14] initial mutations in the cancerogenesis of glioblastoma most likely confer characteristics that help to evade or suppress the immune system or, alternatively, are not “seen” by immune cells in a sufficiently immunogenic context to mount an efficient tumor response. This indicates that the immune system may present as a selection-factor in tumor development, also described by the term immunoediting in analogy to immune receptor editing.[41] Tumor evasion is generally thought to be due to low expression of immunogenic target antigens by tumor cells, rendering them “poorly visible” for T cells, while immunosuppression is defined by multiple mechanisms that inhibit adaptive anti-tumor immune responses.[27,42] Only malignant cells that have gained the ability to suppress and/or evade the immune system survive and evolve to a progressing malignancy. Further, driven by cancer stem- or glioma-initiating cells (GSC) the tumor continuously de-differentiates, differentiates and renews itself, with the result of compromising the immune system`s ability to identify and distinguish malignant from normal brain tissue.[13]

Studies investigating glioblastoma tissue composition revealed that up to 3.2% of cells within a tumor express the pan T cell marker CD3, demonstrating the local presence of immune effector cells.[43] Although a remarkable amount of tumor-infiltrating lymphocytes (TIL) seems to be tumor-antigen specific, most of them do not execute any effector functions or contribute to tumor regression - they virtually have been rendered silent.[44-46] Especially, high-grade gliomas have been shown to suppress, modulate and evade the immune system at multiple levels.[27,41] Hereby the tumor not only alters its immunological appearance, but also plays an active role by manipulating the immune system in its favor.

In the local tumor environment, soluble factors like prostaglandine E_2 (PGE_2), transforming growth factor beta (TGF-β) and interleukin-10 (IL-10) have been described to affect immune function.[44,47,48] PGE_2 inhibits T cell activation, suppresses NK cell activity, downregulates HLA class II expression and induces upregulation of FoxP3 and production of IL-10 in non-T_{reg} cells, steering immune cell composition towards a T_{reg} phenotype.[49-51] TGF-β inhibits T cell proliferation, activation and the function of cytotoxic molecules like perforin or granzymes.[51] In addition, TGF-β impedes DC maturation and antigen presentation, working synergistically with IL-10 in preventing T cell activation and IL-2-induced proliferation.[52,53] TGF-β is partly controlled by adhesion molecule signaling. Integrins like αv, β3 or β5 have been found to control the TGF-β pathway in glioblastoma.[47] Moreover, these soluble factors can be produced by the glioma cells themselves or by local immune cells.[27]

As mentioned above, immune cell infiltration represents a strong feature of gliomas. Almost 25% of all tumor-infiltrating lymphocytes express the regulatory T cell markers CD4, CD25 and FoxP3.[54] Interestingly, the infiltration of CD4+ CD25+ regulatory T cells correlates with glioma malignancy grade,[55] suggesting that malignant progression is closely associated with increased attraction of regulatory T cells. The increased recruitment directly correlates with a negative impact on survival.[42] The suppression of anti-tumor immune responses, however, is not only restricted to the local tumor environment but also appears to extend to a systemic level, since tumor-specific regulatory T cells were also found to be elevated in peripheral blood of glioma patients.[56,57] As a consequence an impaired CD4+ T cell proliferation was observed due to the increased fraction of T_{regs} in glioblastoma.[58,59]

Along with active immunosuppression, glioblastoma mechanisms evolved to evade and counteract CTL-mediated lysis. As mentioned above, a tumor-specific T cell can only identify the tumor if it recognizes tumor antigen bound to surface HLA class I. Mutations within the peptide processing machinery such as altered peptide cleavage or loss of TAP expression, have been described.[60] Further, complete loss of genomic DNA encoding for

certain HLA alleles, HLA gene hypermethylation or alterations in chromatin structure of HLA promoter regions have been demonstrated in glioblastoma.[61] Most efficient for cancer cells is the selective loss of β_2-microglobulin, which abrogates expression of all HLA class I molecules.[62] As a result, 22-43% of tumor cells within a tumor do not express HLA class I, rendering the cancer cells invisible to CTLs.[61,63]

One mechanism of the immune system to counteract tumor-associated loss of HLA class I expression is NK cell-mediated lysis.[64] NK cells expressing the killer immunoglobulin-like receptor (KIR) are activated if the HLA class I molecule is absent.[65] Recently, HLA-G has been described to bind to the inhibitory ILT2 receptor expressed by NK cells, thereby counteracting NK cell activation and CTL-based tumor lysis. HLA-G is expressed by several tumors including glioblastoma, which may contribute to protection from NK cell killing after loss of HLA class I.[66] In addition, malignant gliomas have been shown to also express HLA-E, another non-classical HLA-molecule that inhibits NKG2D-mediated tumor cell lysis.[67-70]

An additional mechanism to hinder immune- and cancer cell interaction is the impairment of adhesion molecule functions. For example, expression of the intercellular adhesion molecule (ICAM-1, CD54) is required for tumor rejection *in vivo.*[71] Since target cell lysis and T cell recognition of TAA presented by HLA class I is dependent on LFA-1/ICAM-1 interaction, the disruption of this mechanism leads to impaired T cell activation.[72,73] Further, extracts from U251 glioma cell lines have shown that extracellular matrix proteins like tenascin-C can inhibit T cell proliferation and cytokine production.[74,75] Glioma cells can also secret glycosaminoglycans, consequently creating a mechanical barrier for immune cell infiltration.[76]

Among other described immunosuppressive pathways like the Fas/FasL- or Galectin-1-interaction,[77,78] we would like to highlight two interesting molecules, which are under current investigation – signal transducer and activator of transcription 3 (STAT3) and indolamine 2,3-dioxygenase (IDO). STAT3 signaling positively regulates production of anti-inflammatory cytokines and anti-apoptotic factors. The observed constitutive overexpression in glioma cells results in the upregulation of anti-apoptotic factors, supposedly conferring an enhanced resistance to chemo-, radio- and CTL-based immunotherapy.[79] Interestingly, in contrast to normal human astrocytes, the silencing of STAT3 expression in glioma cells mediated their apoptosis in the absence of an apoptotic stimulus. Glioma cells can also express IDO, which is an inducible enzyme expressed under the influence of a variety of cytokines, including IFN-γ or TGF-β. IDO catalyzes the degradation of the amino acid tryptophan, which is essential for T cell proliferation.[80] The tryptophan-deprived local tumor

environment can lead to T cell anergy and apoptosis, thereby impairing T cell activation in the local tumor environment.[81,82]

Together, the immunomodulatory mechanisms reviewed above primarily impair T cell activation, cytokine production and execution of effector functions. As a result, the local tumor environment counteracts an effective anti-tumor response. All peptide vaccination strategies will face the challenge of overcoming the local immunosuppression. Although well designed vaccination protocols will presumably be able to recruit large quantities of tumor-specific immune cells, a combined approach that simultaneously tackles the immunosuppressive mechanisms of glioblastoma will most likely be inevitable.

Challenges in antigen selection

Tumor-specific CTLs need to directly interact with the glioma cell to execute their tumor lysing effector functions. This cell-cell-interaction, also called immunological synapse, is mainly defined by the tumor-specific TCR and its cognate tumor-antigen:HLA complex, as well as costimulatory molecules.[83] When searching for the ideal tumor antigen for peptide vaccination, the following considerations have to be taken into account: 1) CTLs can only induce apoptosis and lyse tumor cells, if their specific antigen is naturally presented on a HLA class I molecule. 2) HLA class I molecules are highly polymorphic – different allelic variants bind different sets of peptides.[84] 3) To avoid autoimmunity, the ideal antigen should be expressed exclusively on tumor tissue, or, if an autoimmune response against an antigen should develop, the respective target organ/cell should not lead to major toxicity.

The HLA molecules, which are responsible for presentation of peptides derived from TAAs, are cell surface glycoproteins consisting of an invariant β_2-microglobulin chain and a highly polymorphic heavy chain. They are expressed on the surface of almost all nucleated cells and present peptides of eight to twelve amino acids length to T cells.[84,85] As mentioned above, this peptide:HLA complex is the key structure of the immunological synapse and the level at which the immune system recognizes antigenic structures (see Fig 1 and 2). Proteins derived from all cellular compartments can be degraded into peptides by the proteasome within the cytosol. Afterwards, cytosolic peptides are transported into the lumen of the endoplasmic reticulum (ER) via the transporters associated with antigen processing-1 and -2 (TAP1 and TAP2). Peptide-binding to the HLA class I molecule occurs within the lumen of the ER. The peptide-binding complex includes the chaperones calreticulin, ERp57, and tapasin. Finally, the peptide:HLA complex is transported to the cell surface through the Golgi apparatus.[86]

For surface expression by an HLA molecule, certain amino acids at specific positions of the peptide have to bind into so called “anchoring pockets” of the HLA molecule.[18,87] Depending on its polymorphic regions, every HLA allele requires certain amino acid combinations for their anchoring pockets.[88] If the peptide used for vaccination is not compatible with the HLA haplotype of the patient, it will not be presented to the TCR, and an immune response against the tumor cannot be primed. Most immunotherapeutic approaches in the past have focused on HLA-A*02-restricted peptides since HLA-A*02 is the most prevalent HLA-class I allele in Caucasians,[89] and therefore it has to be taken into consideration, that predominantly HLA-A*02+ patients will benefit from such approaches. By analyzing all HLA class I peptides of an individual patient, one can offer a much broader range of peptides for vaccination, hence limiting the risk of tumor escape.

Possible sources for tumor-associated antigens are differentiation and housekeeping/metabolic antigens, cancer-testis antigens, as well as mutated, differentially spliced, overexpressed or viral antigens.[90] Although the exclusive expression is not required for this approach to work, it certainly reduces the risk of autoimmune reactions. When peptide vaccines derived from overexpressed self-antigens are employed, it appears that the anti-tumor effect correlates with the rate of autoimmunity.[91] For instance, vitiligo is a well-known phenomenon in the immune response to melanoma and is due to shared melanocyte/melanoma antigens – meaning that antigens are presented on both, the healthy melanocyte and the dedifferentiated melanoma cell.[92,93] In the setting of melanoma immunotherapy, the rate of autoimmunity correlates with cancer regression and this side effect occurs in a non-essential organ. Thus, vitiligo poses only a relative concern when being confronted with a life-threatening disease. However, autoimmune reactions against CNS structures are could potentially be far more harmful. A possible solution to this problem might be to primarily focus on mutated peptides. By performing whole genome sequencing of tumor tissue and normal DNA samples, peptides spanning mutated sequences can be defined. As they should only be present on the surface of tumor cells and not on normal tissue, the risk for severe autoimmune reactions and collateral damage is reduced. Furthermore, the tumor antigen should ideally be expressed on strategically important cell subpopulations such as glioma stem cells (GSCs), which play an essential role in tumor biology.[94,95] GSCs represent only a small fraction of the total tumor burden, but these cells are believed to be resistant to chemo- and radiotherapy. Their tumor-initiating capacity is linked to glioblastoma recurrence after cytotoxic treatment.[96]

An important step in understanding T cell activation and adaptive immunity was the discovery of techniques to elute peptides directly out of the HLA binding groove, thereby being able to investigate the natural HLA ligandome presented on the surface of tumors.[97] Protocols such as the “Tübingen approach” have been applied in the past to identify potential tumor antigens.[98] This approach differentially analyzes normal versus tumor tissues, consequently compares mRNA as well as peptide surface expression and includes immunoassays monitoring *in vitro* T cell responses. However, the expression of a given HLA-bound peptide on the surface of the cell does not correlate well with the mRNA expression of its corresponding source protein.[99] In addition, with regards to glioblastoma normal tissue samples usually cannot be provided by the neurosurgeon. Thus, a novel approach well adapted for the highly individual aspects of glioblastoma tumor biology is needed. Two studies analyzed the natural HLA ligandome of glioblastoma using the peptide elution technique in combination with mass spectrometry.[36,100] A number of possible target antigens for peptide vaccination have been described and *in vitro* T cell studies demonstrated solid immune responses. If those ligands will be able to mount an *in vivo* immune response and therefore will be suitable for tumor vaccination is focus of current investigations.

In addition to an HLA class I initiated cytotoxic T cell responses, recent investigations have emphasized the role of CD4+ T_{helper} cells in priming anti-tumor responses after vaccination.[101] In parallel to other complex immune response, i.e. viral infections,[102] CD8+ and CD4+ T_{helper} cell interaction is mandatory for efficient anti-tumor immunity, e.g. by escaping activation-induced CTL cell death or supporting the formation of long-term memory.[103,104] Although the role of CD4+ T_{helper} cells is being discussed controversially,[105] we assume that a vaccinating agent should include tumor-associated peptides, which can bind to HLA class II molecules and promote a tumor-specific involvement of CD4+ T_{helper} cells.

In summary, by analyzing the patient-individual HLA ligandome of freshly resected glioblastoma specimen in contrast to *in vitro* or *in silico* prediction of epitopes, it can be ascertained that a selected candidate peptide is actually naturally presented by the tumor *in vivo*. Whole genome sequencing of tumor and normal DNA allows identification of tumor-specific mutated peptides, thereby reducing the risk of autoimmunity. The aim should be to immunize not only with one, but with a cocktail of peptides adapted to the patient`s HLA haplotype and to the tumor individual mutations in order to reduce the chance of tumor escape. This personalized approach is promising but poses substantial logistical and technical challenges.

Suggestions for a peptide-based treatment protocol

Trying to meet all requirements and challenges of the above described antigen selection for a successful application of peptide-based immunotherapy against glioblastoma, we suggest the following protocol, which is depicted in Figure 3. Directly after initial radiological diagnosis and strong suspicion for a malignant primary brain tumor such as glioblastoma, the required steps for a patient-specific treatment protocol have to be initiated. Together with neurosurgical resection and collection of snap-frozen, as well as vital tumor tissue, PBMCs have to be isolated from EDTA-containing blood tubes or ideally, if possible, by leukapheresis. In addition to standard neuropathological analysis, GSC cultures are initiated and tumor bulk tissue is being snap-frozen for whole tumor HLA ligand elution. Afterwards, the patient will receive standard of care treatment by combined radio- and chemotherapy.[2] During the 6 weeks course of treatment PBMCs are also acquired as healthy tissue control for whole genome sequencing of tumor tissue, including detailed HLA-haplotyping. While GSC cultures are growing a first HLA ligand elution of tumor bulk tissue is performed. The concomitant whole genome sequencing will additionally yield valuable information about tumor-specific mutations. One of the most important steps for the patient-specific targeted vaccine design will then be the antigen selection. First, a comparison of eluted HLA ligands and tumor-specific mutations discovered by the whole genome sequencing will be performed to detect a sufficient amount of suitable antigens. After possible petide modifications to enhance immunogenicity, suitable antigens are synthesized and the patients' T cell response is tested for its reactivity towards the tumor antigens, ideally by at least two different standardized assays, e.g. flow cytometry-based dye dilution, ELISPOT, thymidine incorporation and/or a CTL assay. If a solid immune CD4+ and CD8+ T cell response, ideally against a certain number of HLA class I and –II binding peptides, is detected, final GMP synthesis of the multi-peptide vaccination is initiated. Although there is considerable controversy regarding the interaction of temozolomide with immunotherapeutic protocols, we propose a minimum of 4 weeks between administration of the individually designed peptide-vaccine and the last dose of chemotherapy. Subsequently further PBMC samples should be collected and immune response should be monitored to determine T cell expansion as well as the optimal time point for re-boosting with the patient-suited multi peptide vaccine. The above strategy is in our mind an ideal one, however, as stated before, it is logistically very challenging in its implementation and costly.

SUMMARY, OUTLOOK AND FUTURE CHALLENGES

In this review we discussed challenges for peptide vaccination protocols in glioblastoma. One of the most important issues to be overcome is the tumor heterogeneity at

a molecular and cellular level, which is presumably driven by GSCs along with a naturally transforming cell population and maintained by the high prevalence of new mutations within a fast growing tumor. This inter- and even intra-individual heterogeneity complicates the selection of appropriate targets for vaccination and thereby also the development of “off-the-shelf” approaches. Therefore, patient-individual treatment protocols are needed. Immunosuppressive properties of glioblastoma are mainly the result of cooperating processes of selection and mutation, which is summarized under the term immunoediting.[41] Due to the large variety of pathways employed by the tumor to evade, suppress and manipulate the immune system, especially in the local tumor environment, peptide vaccination strategies have to find appropriate ways to circumvent immunosuppresion to be able to translate into clinical success. Since murine studies have produced encouraging results of long-term immunity,[106] the goal of clinical immunotherapy has to remain achieving complete tumor regression. The following strategies represent options to overcome the abovementioned hurdles and pave the way to successful immunotherapy in glioblastoma.

To counteract heterogeneity and circumvent tumor-induced suppression of antigen-specific T cell responses, tumor vaccines should consist of multiple peptides that ideally should elicit efficient CD4+ and CD8+ T cell responses. Targeting multiple tumor-associated antigens will enhance the magnitude of immune response and decrease the chance of tumor escape by positive clonal selection of antigen-loss variants.[90] As described above, given the increasing evidence for a central role of GSCs in glioblastoma, a multi-peptide vaccine should also target stem cell-associated antigens in order to eliminate potential sources for recurrences. One future challenge will be the development of a GSC-targeting immunotherapy that avoids an autoimmune response against normal adult neural stem/progenitor cells.[96] These cells share a similar antigen profile with GSCs and potential and mediate an important role after chemo- and radiotherapy-induced tissue destruction. They also have been proposed to inhibit glioblastoma growth by inducing cancer cell apoptosis.[107] Furthermore, to enhance anti-tumor efficacy, vaccines have to include peptides eluted from HLA class II for the purpose of eliciting a supportive CD4+ T cell expansion.[108] CD4+ T_{Helper} cells orchestrate initiation of an adaptive immune response and augment CTL expansion. Murine data has confirmed that CD4+ T cells are essential to generate long-term tumor immunity.[106,109] Peptide modifications that enhance binding properties to HLA class I and -II, without sacrificing tumor-antigen specificity can facilitate immunogenicity and induce a greater TCR diversity. In addition, the targeting peptide sequence can be incorporated into long peptides to avoid unspecific peptide binding to unoccupied HLA molecules, thereby improving natural uptake and processing of the peptide resulting in enhanced presentation on antigen presenting cells.[110] Modifications like myristilation, amino acid substitution,

backbone reduction, partial retro-inversion or terminal alterations of the peptide have been proposed to further enhance immunogenicity.[111]

To discuss the challenge of possible targets to clinically counteract tumor immunosuppressive in the local tumor environment as well as at the systemic level, two principles of adjuvants to peptide vaccination protocols have to be considered – relieving immunosuppression and boosting the induced anti-tumor response. Regarding antagonizing immunosuppression, most approaches aim to deplete regulatory T cells prior to immunization. Clinical trials in humans primarily used agents like anti-CD25 antibodies[112] or low-dose cyclophosphamide to achieve a depletion of CD4+ CD25+ regulatory T cells.[29] A multitude of approaches to selectively target regulatory T cells are under investigation,[113] e.g. toxin-fused IL-2 to target the CD25 epitope.[114] With removal of one of the central mediators within the local tumor environment, less soluble factors like IL-10 and TGF-β are secreted and more IL-2 is available for expansion of tumor-specific CTLs. Unfortunately, since T cells also upregulate the high-affinity IL-2 receptor alpha-chain (CD25) upon activation, administration of an anti-CD25-specific antibody depletes antigen-activated T cells, presumably weakening the intrinsic anti-tumor response of the host. Therefore, additional studies are needed that will yield valuable results about timing and improvement of selectivity of lymphodepletion. Another strategy is to complement peripheral delivery of DCs with intratumoral (IT) injections of peptide-pulsed DCs (pDC). Mice treated with IT-pDC plus subcutaneous (SC) pDC survived longer than mice treated by SC-pDC only. Further, the intratumoral injection of pDCs resulted in a significant decrease in FoxP3+ regulatory T cells, and an increase in CD8+ T cells, indicating a change in the local tumor environment favoring anti-tumor responses.[115] Besides the use of antibodies to deplete T_{regs} and the local administration of cells facilitating immune activation, new compound release systems, directly applied to the tumor bed after surgical resection, could be able to deliver antibodies or cytokines to defuse the local immunosuppressive milieu of the tumor. Inspired by the gliadel™ principle, where biodegradable polymer wafers are implanted after tumor removal to continuously release the chemotherapeutic agents carmustine,[116] neurosurgical interventions could include placement of immunomodulatory agents directly to the tumor bed.

Besides directly focusing on the presence of regulatory T cells, the inhibition of soluble factors, especially TGF-β might facilitate improvement to an effective immune response. In that regard, a randomized study exploring the local delivery of TGF-β antisense oligonucleotides via catheter system confirmed the feasibility of this approach, but did not examine target inhibition and did not confirm its efficacy.[117] Current approaches to inhibit

TGF-β pathway activation focus on small molecule inhibitors of Alk5, the major TGF-β receptor I, e.g., using LY2157299.[118]

To support the priming and execution of the peripherally activated anti-tumor response new compound release systems like microparticles containing cytokines like IL-7,[119], IL-15, IL-21, IFN-γ, antibodies intercepting IL-10 or small molecules enhancing APC function, T cell activation and proliferation, breaking down the barriers for an efficient tumor cell lysis by glioma-specific T cells are under investigation. Additionally boosting the clonal expansion can be achieved by TLR agonists like poly I:C or imiquimod.[120]

The modality of peptide vaccines offers many additional therapeutic options besides targeting the tumor cells directly. Immunization with antigens exclusively expressed during angiogenesis could, for example, limit the supply of nutrition to the expanding tumor.[121,122] Further, radio- and chemotherapy applies selective pressure on the tumor. This has been shown to result in mutations conferring properties of radio- and chemotherapy resistance to the tumor. The proteins mediating mechanisms of resistance now may result in new target antigens for immunotherapy, in the sense that peptide vaccines could not only constitute as an independent treatment approach,[123] but also serve as a synergistic tool supporting other treatment modalities. Individualized peptide-vaccination is therefore not only able to target patient-specific mutations and cell subpopulations, but also has the potential to be combined with other treatment modalities such as radio- and chemotherapy and represents an optimal complementation to neurosurgical treatment. Since immunotherapy is unlikely to be able to eradicate a large established tumor burden by itself, it is depending, at least for now, on neurosurgical resection for both tissue sampling and reduction of tumor load.

CONCLUSION

The heterogeneity and the transforming nature of glioblastoma as well as tumor-associated immunosuppression and immune evasion mechanisms pose a major challenge to treating physicians. Since conventional treatment options such as surgery, radio- and chemotherapy failed to profoundly improve overall survival; new treatment modalities are desperately needed. Immunotherapy offers high specificity and appears to be one of the most promising targeted approaches. We suggest a protocol to define tumor-associated antigens for peptide-based immunotherapy. Key elements of our approach are patient-individual screening for antigens, the use of multiple peptides covering all HLA alleles of the patient, the inclusion of both HLA class I and -II peptides, the focus on tumor-specific mutated peptides, and the definition of HLA ligands eluted from GSCs. Suitable antigenic

peptides can then be used for peptide vaccination, but also for the loading of dendritic cells or for priming CTLs prior to an adoptive transfer to the patient.

FIGURE LEGENDS

Figure 1: Key players in immunotherapy

The schematic overview depicts the idealized vaccination-induced peripheral priming of a tumor-specific immune response. After peptide uptake and processing peripheral antigen presenting cells, especially dendritic cells, present the antigen via HLA-class I and -II molecules to tumor-specific T cells. After TCR-peptide:HLA and costimulatory molecule-induced activation, T cells proliferate and execute their effector functions. CD4+ T_{helper} cells promote the perpetuation of the mounted immune response by activating local antigen presenting cells (APC), while CD8+ cytotoxic T cells directly lyse tumor cells through induction of apoptosis by, i.e. perforin, granzyme and granulysin. Glioblastoma cells try to evade immune-mediated lysis by attracting regulatory T cells, thereby promoting an anti-inflammatory milieu and manipulating the local tumor environment in their

Figure 2: HLA presentation of mutated peptides and CD8+ T cell interaction

Cellular proteins of glioblastoma cells – some of them carrying tumor-specific mutated sequences (shown in red) – are degraded into peptides by the proteasome within the cytosol. These peptides are transported into the lumen of the endoplasmic reticulum via the transporter associated with antigen processing (TAP) and loaded onto HLA class I molecules. The peptide:HLA complex is transported to the cell surface via the Golgi apparatus and presents potential tumor antigens to CD8+ cytotoxic T cells, which recognize the antigen via their cognate T cell receptor (TCR) .

Figure 3: Personalized peptide-based treatment protocol

A suggestion for a individualized peptide-based glioblastoma vaccination algorhythm. PBMCs = peripheral blood mononuclear cells; GSCs = glioma stem cells; HLA = human leukocyte antigen; GMP = good manufacturing practice

REFERENCES

1. Preusser M, de Ribaupierre S, Wohrer A, Erridge SC, Hegi M, Weller M, Stupp R. Current concepts and management of glioblastoma. Ann Neurol. 2011;70:9-21.

2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987-96.

3. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10:459-66.

4. Ohgaki H, Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol. 2005;64:479-89.

5. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, Hegi ME. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol. 2010;6:39-51.

6. Curran WJ, Jr., Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst. 1993;85:704-10.

7. Choucair AK, Levin VA, Gutin PH, Davis RL, Silver P, Edwards MS, Wilson CB. Development of multiple lesions during radiation therapy and chemotherapy in patients with gliomas. J Neurosurg. 1986;65:654-8.

8. Pilkington GJ, Bjerkvig R, De Ridder L, Kaaijk P. In vitro and in vivo models for the study of brain tumour invasion. Anticancer Res. 1997;17:4107-9.

9. Giese A, Kluwe L, Laube B, Meissner H, Berens ME, Westphal M. Migration of human glioma cells on myelin. Neurosurgery. 1996;38:755-64.

10. Pichlmeier U, Bink A, Schackert G, Stummer W. Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro Oncol. 2008;10:1025-34.

11. Squatrito M, Holland EC. DNA damage response and growth factor signaling pathways in gliomagenesis and therapeutic resistance. Cancer Res. 2011;71:5945-9.

12. Beier D, Schulz JB, Beier CP. Chemoresistance of glioblastoma cancer stem cells--much more complex than expected. Mol Cancer. 2011;10:128.

13. Bonavia R, Inda MM, Cavenee WK, Furnari FB. Heterogeneity maintenance in glioblastoma: a social network. Cancer Res. 2011;71:4055-60.

14. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235-71.

15. Beckman RA, Loeb LA. Genetic instability in cancer: theory and experiment. Semin Cancer Biol. 2005;15:423-35.

16. Vauleon E, Avril T, Collet B, Mosser J, Quillien V. Overview of cellular immunotherapy for patients with glioblastoma. Clin Dev Immunol. 2010;2010.

17. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51-72.

18. Fooksman DR, Vardhana S, Vasiliver-Shamis G, Liese J, Blair DA, Waite J, et al. Functional anatomy of T cell activation and synapse formation. Annu Rev Immunol. 2010;28:79-105.

19. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol. 2002;20:621-67.

20. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol. 2012;12:557-69.

21. Yamanaka R, Itoh K. Peptide-based immunotherapeutic approaches to glioma: a review. Expert Opin Biol Ther. 2007;7:645-9.

22. Kronik N, Kogan Y, Elishmereni M, Halevi-Tobias K, Vuk-Pavlovic S, Agur Z. Predicting outcomes of prostate cancer immunotherapy by personalized mathematical models. PLoS One. 2010;5:e15482.

23. Tacken PJ, de Vries IJ, Torensma R, Figdor CG. Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting. Nat Rev Immunol. 2007;7:790-802.

24. Ludewig B, Ochsenbein AF, Odermatt B, Paulin D, Hengartner H, Zinkernagel RM. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. The Journal of experimental medicine. 2000;191:795-804.

25. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411-22.
26. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004;10:909-15.

27. Albesiano E, Han JE, Lim M. Mechanisms of local immunoresistance in glioma. Neurosurg Clin N Am. 2010;21:17-29.

28. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364:2119-27.

29. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med. 2012.

30. Choi BD, Archer GE, Mitchell DA, Heimberger AB, McLendon RE, Bigner DD, Sampson JH. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol. 2009;19:713-23.

31. Hegi ME, Rajakannu P, Weller M. Epidermal growth factor receptor: a re-emerging target in glioblastoma. Curr Opin Neurol. 2012;25:774-9.

32. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF, Collins VP. Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their expression in human gliomas in vivo. Cancer Res. 1991;51:2164-72.

33. Gan HK, Kaye AH, Luwor RB. The EGFRvIII variant in glioblastoma multiforme. J Clin Neurosci. 2009;16:748-54.

34. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE, 2nd, Lally-Goss D, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Mol Cancer Ther. 2009;8:2773-9.

35. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol. 2010;28:4722-9.

36. Dutoit V, Herold-Mende C, Hilf N, Schoor O, Beckhove P, Bucher J, et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. Brain. 2012;135:1042-54.

37. Ebben JD, Rocque BG, Kuo JS. Tumour vaccine approaches for CNS malignancies: progress to date. Drugs. 2009;69:241-9.

38. Lohr J, Ratliff T, Huppertz A, Ge Y, Dictus C, Ahmadi R, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. Clin Cancer Res. 2011;17:4296-308.

39. Yang I, Tihan T, Han SJ, Wrensch MR, Wiencke J, Sughrue ME, Parsa AT. CD8+ T-cell infiltrate in newly diagnosed glioblastoma is associated with long-term survival. J Clin Neurosci. 2010;17:1381-5.

40. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646-74.

41. Dunn GP, Fecci PE, Curry WT. Cancer immunoediting in malignant glioma. Neurosurgery. 2012;71:201-22; discussion 22-3.

42. Wainwright DA, Balyasnikova IV, Chang AL, Ahmed AU, Moon KS, Auffinger B, et al. IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. Clin Cancer Res. 2012;18:6110-21.

43. Waziri A, Killory B, Ogden AT, 3rd, Canoll P, Anderson RC, Kent SC, et al. Preferential in situ CD4+CD56+ T cell activation and expansion within human glioblastoma. J Immunol. 2008;180:7673-80.

44. Crane CA, Ahn BJ, Han SJ, Parsa AT. Soluble factors secreted by glioblastoma cell lines facilitate recruitment, survival, and expansion of regulatory T cells: implications for immunotherapy. Neuro Oncol. 2012;14:584-95.

45. Kim YH, Jung TY, Jung S, Jang WY, Moon KS, Kim IY, et al. Tumour-infiltrating T-cell subpopulations in glioblastomas. Br J Neurosurg. 2012;26:21-7.

46. Roszman TL, Brooks WH. Immunobiology of primary intracranial tumours. III. Demonstration of a qualitative lymphocyte abnormality in patients with primary brain tumours. Clin Exp Immunol. 1980;39:395-402.

47. Roth P, Silginer M, Goodman SL, Hasenbach K, Thies S, Maurer G, et al. Integrin control of the transforming growth factor-beta pathway in glioblastoma. Brain. 2013;136:564-76.

48. Seoane J. TGFbeta and cancer initiating cells. Cell Cycle. 2009;8:3787-8.

49. Wojtowicz-Praga S. Reversal of tumor-induced immunosuppression: a new approach to cancer therapy. J Immunother. 1997;20:165-77.

50. Baxevanis CN, Reclos GJ, Gritzapis AD, Dedousis GV, Missitzis I, Papamichail M. Elevated prostaglandin E2 production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. Cancer. 1993;72:491-501.

51. Zuber P, Kuppner MC, De Tribolet N. Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. Eur J Immunol. 1988;18:1623-6.

52. Liu VC, Wong LY, Jang T, Shah AH, Park I, Yang X, et al. Tumor evasion of the immune system by converting CD4+CD25- T cells into CD4+CD25+ T regulatory cells: role of tumor-derived TGF-beta. J Immunol. 2007;178:2883-92.

53. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685-711.

54. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. Neuro Oncol. 2006;8:234-43.

55. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, Yang DS, Sun W, Qiao W, et al. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res. 2008;14:5166-72.

56. Sonabend AM, Rolle CE, Lesniak MS. The role of regulatory T cells in malignant glioma. Anticancer Res. 2008;28:1143-50.

57. Elliott LH, Brooks WH, Roszman TL. Cytokinetic basis for the impaired activation of lymphocytes from patients with primary intracranial tumors. J Immunol. 1984;132:1208-15.

58. Grauer OM, Nierkens S, Bennink E, Toonen LW, Boon L, Wesseling P, et al. CD4+FoxP3+ regulatory T cells gradually accumulate in gliomas during tumor growth and efficiently suppress antiglioma immune responses in vivo. Int J Cancer. 2007;121:95-105.

59. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, et al. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res. 2006;66:3294-302.

60. Facoetti A, Nano R, Zelini P, Morbini P, Benericetti E, Ceroni M, et al. Human leukocyte antigen and antigen processing machinery component defects in astrocytic tumors. Clin Cancer Res. 2005;11:8304-11.

61. Yeung JT, Hamilton RL, Ohnishi K, Ikeura M, Potter DM, Nikiforova MN, et al. LOH in the HLA Class I Region at 6p21 Is Associated with Shorter Survival in Newly Diagnosed Adult Glioblastoma. Clin Cancer Res. 2013;19:1816-26.

62. Restifo NP, Marincola FM, Kawakami Y, Taubenberger J, Yannelli JR, Rosenberg SA. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. J Natl Cancer Inst. 1996;88:100-8.

63. Zagzag D, Salnikow K, Chiriboga L, Yee H, Lan L, Ali MA, et al. Downregulation of major histocompatibility complex antigens in invading glioma cells: stealth invasion of the brain. Lab Invest. 2005;85:328-41.

64. Lanier LL. NK cell recognition. Annu Rev Immunol. 2005;23:225-74.

65. Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol. 2009;27:83-117.

66. Wiendl H, Mitsdoerffer M, Hofmeister V, Wischhusen J, Bornemann A, Meyermann R, et al. A functional role of HLA-G expression in human gliomas: an alternative strategy of immune escape. J Immunol. 2002;168:4772-80.

67. Wischhusen J, Friese MA, Mittelbronn M, Meyermann R, Weller M. HLA-E protects glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo. J Neuropathol Exp Neurol. 2005;64:523-8.

68. Mittelbronn M, Simon P, Loffler C, Capper D, Bunz B, Harter P, et al. Elevated HLA-E levels in human glioblastomas but not in grade I to III astrocytomas correlate with infiltrating CD8+ cells. J Neuroimmunol. 2007;189:50-8.

69. Wolpert F, Roth P, Lamszus K, Tabatabai G, Weller M, Eisele G. HLA-E contributes to an immune-inhibitory phenotype of glioblastoma stem-like cells. J Neuroimmunol. 2012;250:27-34.

70. Friese MA, Platten M, Lutz SZ, Naumann U, Aulwurm S, Bischof F, et al. MICA/NKG2D-mediated immunogene therapy of experimental gliomas. Cancer Res. 2003;63:8996-9006.

71. Gomez GG, Kruse CA. Mechanisms of malignant glioma immune resistance and sources of immunosuppression. Gene Ther Mol Biol. 2006;10:133-46.

72. Schiltz PM, Gomez GG, Read SB, Kulprathipanja NV, Kruse CA. Effects of IFN-gamma and interleukin-1beta on major histocompatibility complex antigen and intercellular adhesion

molecule-1 expression by 9L gliosarcoma: relevance to its cytolysis by alloreactive cytotoxic T lymphocytes. J Interferon Cytokine Res. 2002;22:1209-16.

73. Fiore E, Fusco C, Romero P, Stamenkovic I. Matrix metalloproteinase 9 (MMP-9/gelatinase B) proteolytically cleaves ICAM-1 and participates in tumor cell resistance to natural killer cell-mediated cytotoxicity. Oncogene. 2002;21:5213-23.

74. Hemesath TJ, Marton LS, Stefansson K. Inhibition of T cell activation by the extracellular matrix protein tenascin. J Immunol. 1994;152:5199-207.

75. Parekh K, Ramachandran S, Cooper J, Bigner D, Patterson A, Mohanakumar T. Tenascin-C, over expressed in lung cancer down regulates effector functions of tumor infiltrating lymphocytes. Lung Cancer. 2005;47:17-29.

76. Dick SJ, Macchi B, Papazoglou S, Oldfield EH, Kornblith PL, Smith BH, Gately MK. Lymphoid cell-glioma cell interaction enhances cell coat production by human gliomas: novel suppressor mechanism. Science. 1983;220:739-42.

77. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, et al. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. Cancer Cell. 2004;5:241-51.

78. Badie B, Schartner J, Prabakaran S, Paul J, Vorpahl J. Expression of Fas ligand by microglia: possible role in glioma immune evasion. J Neuroimmunol. 2001;120:19-24.

79. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, et al. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med. 2004;10:48-54.

80. Miyazaki T, Moritake K, Yamada K, Hara N, Osago H, Shibata T, et al. Indoleamine 2,3-dioxygenase as a new target for malignant glioma therapy. Laboratory investigation. J Neurosurg. 2009;111:230-7.

81. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9:1269-74.

82. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. Cancer Res. 2012;72:5435-40.

83. Smith-Garvin JE, Koretzky GA, Jordan MS. T cell activation. Annu Rev Immunol. 2009;27:591-619.

84. Rammensee HG, Falk K, Rotzschke O. Peptides naturally presented by MHC class I molecules. Annu Rev Immunol. 1993;11:213-44.

85. Stevanovic S, Schild H. Quantitative aspects of T cell activation--peptide generation and editing by MHC class I molecules. Seminars in immunology. 1999;11:375-84.

86. Yewdell JW, Haeryfar SM. Understanding presentation of viral antigens to CD8+ T cells in vivo: the key to rational vaccine design. Annu Rev Immunol. 2005;23:651-82.

87. Marrack P, Rubtsova K, Scott-Browne J, Kappler JW. T cell receptor specificity for major histocompatibility complex proteins. Curr Opin Immunol. 2008;20:203-7.

88. Rammensee HG, Falk K, Rotzschke O. MHC molecules as peptide receptors. Curr Opin Immunol. 1993;5:35-44.

89. Ellis JM, Henson V, Slack R, Ng J, Hartzman RJ, Katovich Hurley C. Frequencies of HLA-A2 alleles in five U.S. population groups. Predominance Of A*02011 and identification of HLA-A*0231. Hum Immunol. 2000;61:334-40.

90. Rammensee HG, Weinschenk T, Gouttefangeas C, Stevanovic S. Towards patient-specific tumor antigen selection for vaccination. Immunol Rev. 2002;188:164-76.

91. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298:850-4.

92. Byrne KT, Turk MJ. New perspectives on the role of vitiligo in immune responses to melanoma. Oncotarget. 2011;2:684-94.

93. Rosenberg SA, White DE. Vitiligo in patients with melanoma: normal tissue antigens can be targets for cancer immunotherapy. J Immunother Emphasis Tumor Immunol. 1996;19:81-4.

94. Romero P, Cerottini JC, Speiser DE. Monitoring tumor antigen specific T-cell responses in cancer patients and phase I clinical trials of peptide-based vaccination. Cancer Immunol Immunother. 2004;53:249-55.

95. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature. 2004;432:396-401.

96. Lamszus K, Gunther HS. Glioma stem cells as a target for treatment. Target Oncol. 2010;5:211-5.

97. Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature. 1991;351:290-6.

98. Singh-Jasuja H, Emmerich NP, Rammensee HG. The Tubingen approach: identification, selection, and validation of tumor-associated HLA peptides for cancer therapy. Cancer Immunol Immunother. 2004;53:187-95.

99. Weinzierl AO, Lemmel C, Schoor O, Muller M, Kruger T, Wernet D, et al. Distorted relation between mRNA copy number and corresponding major histocompatibility complex ligand density on the cell surface. Molecular & cellular proteomics : MCP. 2007;6:102-13.

100. Neidert MC, Schoor O, Trautwein C, Trautwein N, Christ L, Melms A, et al. Natural HLA class I ligands from glioblastoma: extending the options for immunotherapy. Journal of neuro-oncology. 2013;111:285-94.

101. Kennedy R, Celis E. Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunol Rev. 2008;222:129-44.

102. Zajac AJ, Murali-Krishna K, Blattman JN, Ahmed R. Therapeutic vaccination against chronic viral infection: the importance of cooperation between CD4+ and CD8+ T cells. Curr Opin Immunol. 1998;10:444-9.

103. Kennedy R, Celis E. T helper lymphocytes rescue CTL from activation-induced cell death. J Immunol. 2006;177:2862-72.

104. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. Nature. 2003;421:852-6.

105. Assudani D, Cho HI, DeVito N, Bradley N, Celis E. In vivo expansion, persistence, and function of peptide vaccine-induced CD8 T cells occur independently of CD4 T cells. Cancer Res. 2008;68:9892-9.

106. Newcomb EW, Demaria S, Lukyanov Y, Shao Y, Schnee T, Kawashima N, et al. The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. Clin Cancer Res. 2006;12:4730-7.

107. Glass R, Synowitz M, Kronenberg G, Walzlein JH, Markovic DS, Wang LP, et al. Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. J Neurosci. 2005;25:2637-46.

108. Accolla RS, Tosi G. Optimal MHC-II-restricted tumor antigen presentation to CD4+ T helper cells: the key issue for development of anti-tumor vaccines. J Transl Med. 2012;10:154.

109. Ochiai H, Archer GE, Herndon JE, 2nd, Kuan CT, Mitchell DA, Bigner DD, et al. EGFRvIII-targeted immunotoxin induces antitumor immunity that is inhibited in the absence of CD4+ and CD8+ T cells. Cancer Immunol Immunother. 2008;57:115-21.

110. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008;8:351-60.

111. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov. 2007;6:404-14.

112. Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. Cancer Res. 1999;59:3128-33.

113. Nishikawa H, Sakaguchi S. Regulatory T cells in tumor immunity. Int J Cancer. 2010;127:759-67.

114. Nizard P, Gross DA, Babon A, Chenal A, Beaumelle B, Kosmatopoulos K, Gillet D. Anchoring cytokines to tumor cells for the preparation of anticancer vaccines without gene transfection in mice. J Immunother. 2003;26:63-71.

115. Pellegatta S, Poliani PL, Stucchi E, Corno D, Colombo CA, Orzan F, et al. Intra-tumoral dendritic cells increase efficacy of peripheral vaccination by modulation of glioma microenvironment. Neuro Oncol. 2010;12:377-88.

116. Brem H, Gabikian P. Biodegradable polymer implants to treat brain tumors. J Control Release. 2001;74:63-7.

117. Bogdahn U, Hau P, Stockhammer G, Venkataramana NK, Mahapatra AK, Suri A, et al. Targeted therapy for high-grade glioma with the TGF-beta2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. Neuro Oncol. 2011;13:132-42.

118. Rodon J, Sepúlveda JM, Azaro A, Calvo E, Seoane J, Brana et al. Integrated data review of the first-in-human dose (FHD) study evaluating safety, pharmacokinetics (PK), and pharmacodynamics (PD) of the oral transforming growth factor-beta (TGF-ß) receptor I kinase inhibitor, LY2157299 monohydrate (LY). ASCO Annual Meeting 2013.

119. Wang NX, Bazdar DA, Sieg SF, von Recum HA. Microparticle delivery of Interleukin-7 to boost T-cell proliferation and survival. Biotechnol Bioeng. 2012;109:1835-43.

120. Warger T, Schild H, Rechtsteiner G. Initiation of adaptive immune responses by transcutaneous immunization. Immunol Lett. 2007;109:13-20.

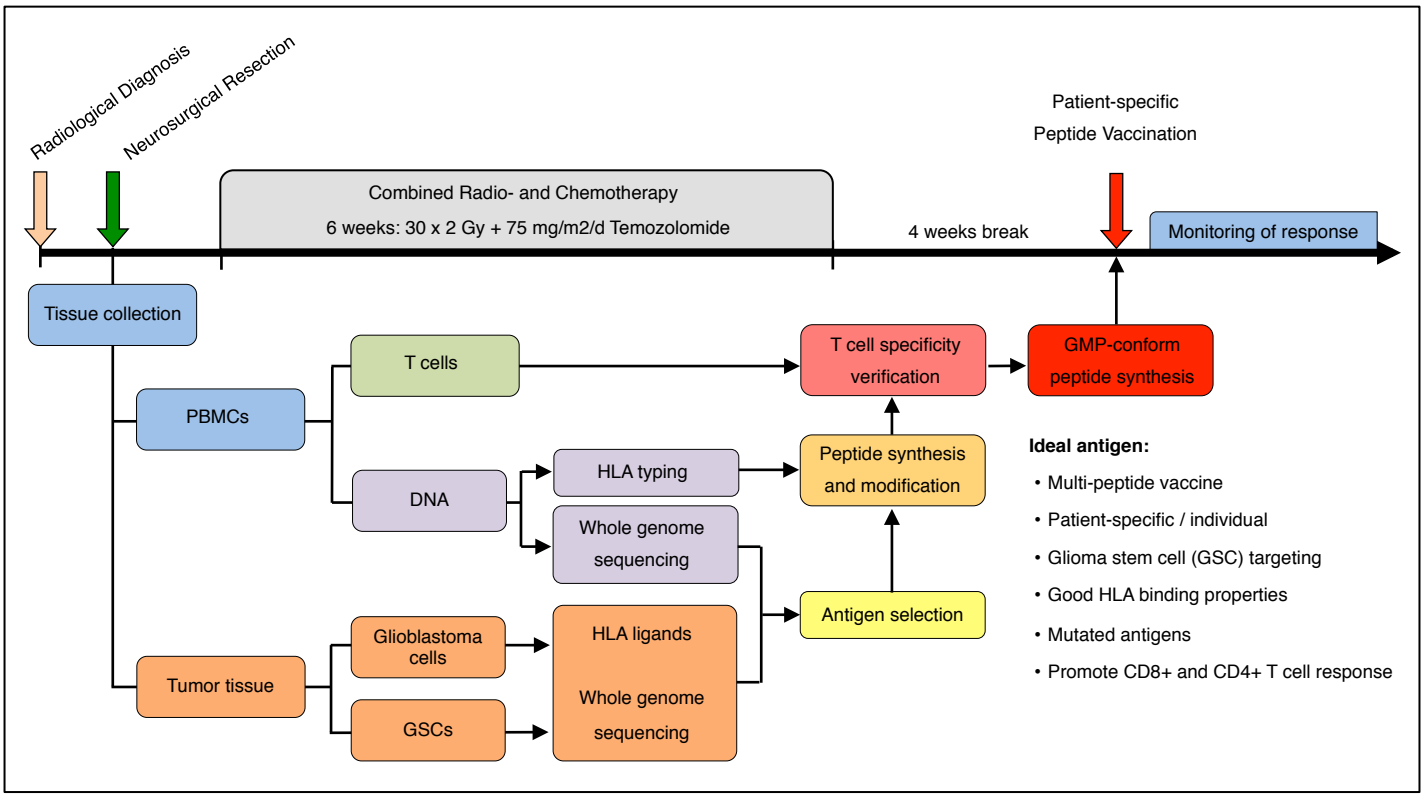
121. Pan J, Jin P, Yan J, Kabelitz D. Anti-angiogenic active immunotherapy: a new approach to cancer treatment. Cancer Immunol Immunother. 2008;57:1105-14.

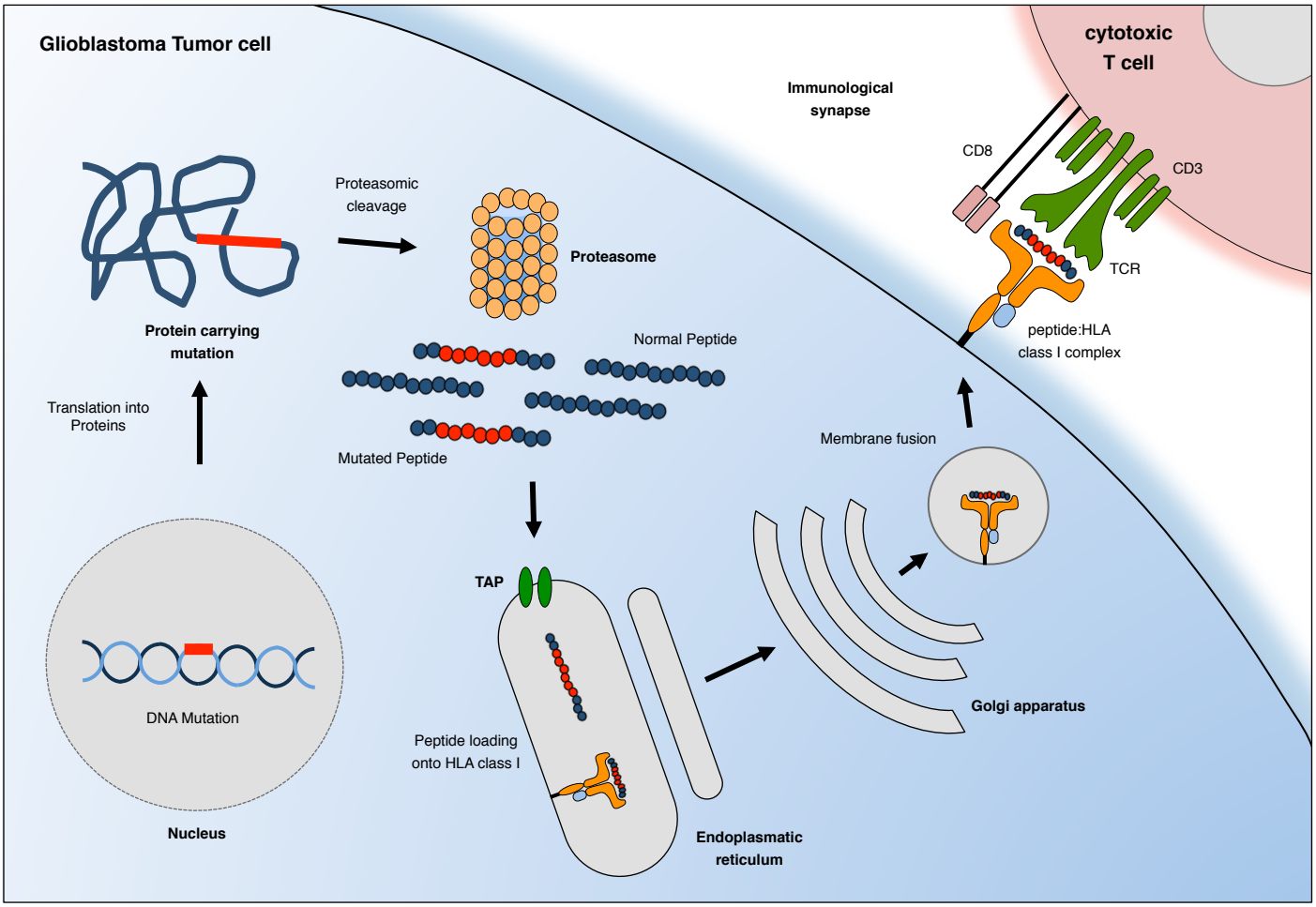
122. Hou J, Tian L, Wei Y. Cancer immunotherapy of targeting angiogenesis. Cell Mol Immunol. 2004;1:161-6.

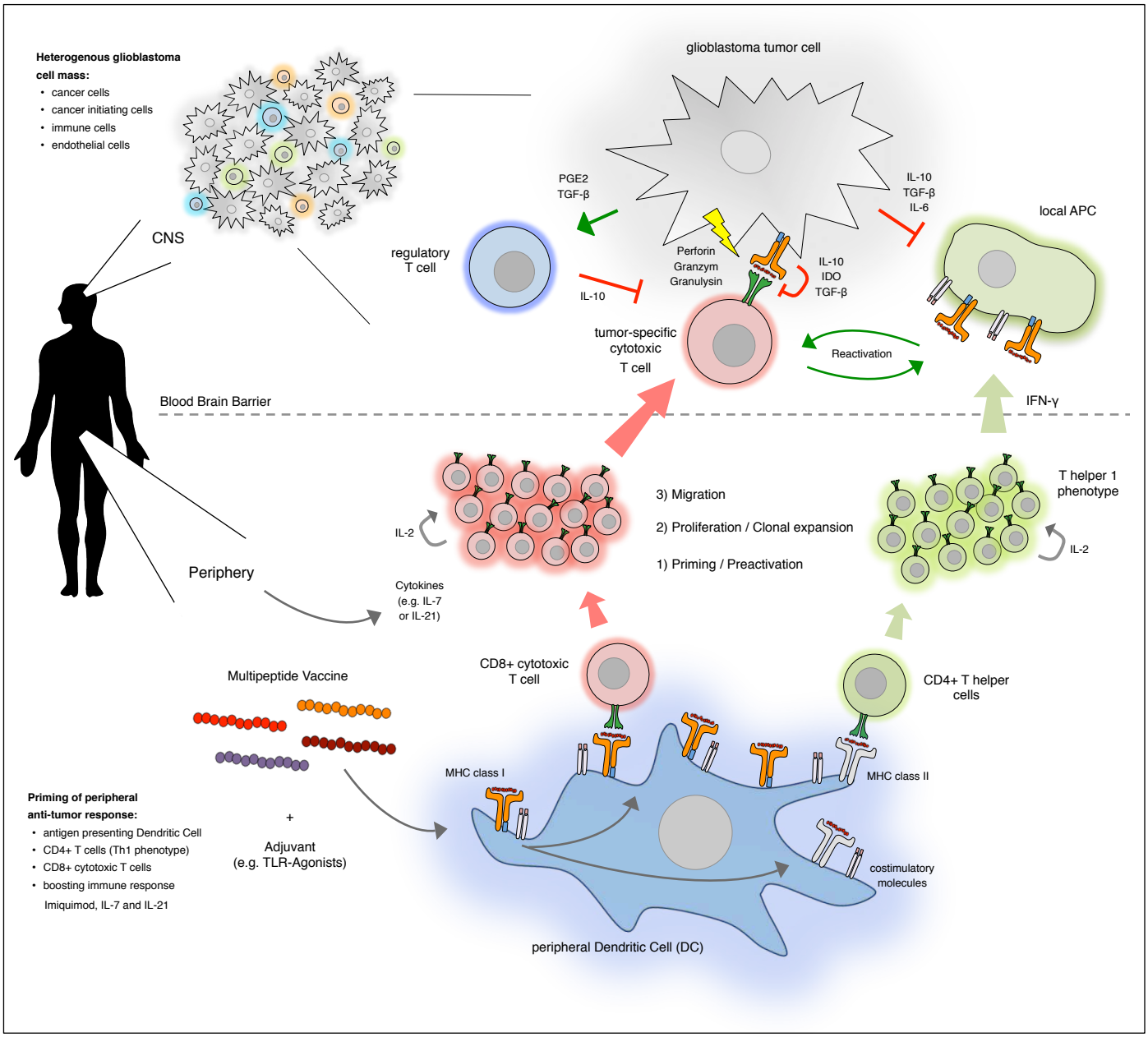
123. Liu G, Akasaki Y, Khong HT, Wheeler CJ, Das A, Black KL, Yu JS. Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. Oncogene. 2005;24:5226-34.

Table 1: Ongoing clinical trials involving peptide vaccines targeting glioblastoma according to the database of ClinicalTrials.gov

Phase	Estimated Completion / Size	Sponsor	Protocol
Phase I/II ID: NCT00293423	Date: 12/2013 Enrollment: 50	University of California, San Francisco	Trial of Heat Shock Protein Peptide Complex-96 (HSPPC-96) vaccine for patients with recurrent high-grade glioma.
Phase I ID: NCT01403285	Date: 03/2014 Enrollment: 25	immatics Biotechnologies GmbH	Trial of peptide-based glioma vaccine IMA950 in patients with glioblastoma. IMA950: multi-peptide vaccine containing 11 tumor-associated peptides (TUMAPs) found in a majority of glioblastomas.
Phase I ID: NCT01250470	Date: 09/2014 Enrollment: 9	Roswell Park Cancer Institute	Study of safety, tolerability and immunological effects of SVN53-67/M57-KLH in patients with Survivin-positive malignant gliomas.
Phase I ID: NCT00626015	Date: 11/2013 Enrollment: 20	John Sampson, Duke University Medical Center	Chemotherapy, Radiation Therapy, and Vaccine (PEP-3-KLH) Therapy With Basiliximab in Treating Patients With Glioblastoma Multiforme That Has Been Removed by Surgery
Phase I/II ID: NCT01920191	Date: 08/2014 Enrollment: 16	University Hospital, Geneva	Study of intradermal IMA950 peptide-based vaccine adjuvanted with intramuscular Poly-ICLC in combination with temozolomide in newly diagnosed HLA-A2 glioblastoma patients.
Phase II ID: NCT01814813	Date: 04/2016 Enrollment: 222	Alliance for Clinical Trials in Oncology	Randomized trial comparing the efficacy of Heat Shock Protein-Peptide Complex-96 (HSPPC-96) vaccine given with Bevacizumab versus Bevacizumab alone in the treatment of surgically resectable recurrent glioblastoma. Experimental: Arm 1, HSPPC-96, concomitant Bevacizumab. Experimental: Arm 2, HSPPC-96 with Bevacizumab at progression Active Comparator: Arm 3, Bevacizumab
Phase II ID: NCT00905060	Date: 01/2014 Enrollment: 555	University of California, San Francisco	Multi-center, single arm investigation of HSPPC-96 vaccine with Temozolomide in patients with newly diagnosed glioblastoma.
Phase II ID: NCT00643097	Date: 06/2016 Enrollment: 48	John Sampson, Duke University Medical Center	A Complementary trial of an immunotherapy vaccine against tumor-specific EGFRvIII. Arm I: Patients receive PEP-3-KLH conjugate vaccine and Sargramostim (GM-CSF) intradermally on days 1, 15, and 29 and then monthly in the absence of disease progression or unacceptable toxicity. Arm II: Patients receive placebo vaccine intradermally on days 1, 15, and 29. Patients then receive PEP-3-KLH conjugate vaccine and GM-CSF monthly in the absence of disease progression or unacceptable toxicity.
Phase II ID: NCT01498328	Date: 06/2015 Enrollment: 168	Celldex Therapeutics	A Phase II Study of Rindopepimut/GM-CSF in patients with relapsed EGFRvIII-positive glioblastoma (ReACT). Experimental: Group 1a: Bevacizumab Naïve with Bevacizumab + rindopepimut. Experimental: Group 1b: Bevacizumab Naïve with Bevacizumab + KLH control Experimental: Group 2 and 2C: Refractory to Bevacizumab
Phase III ID: NCT01498328	Date: 11/2016 Enrollment: 440	Celldex Therapeutics	An international, randomized, double-blind, controlled study of Rindopepimut/GM-CSF with adjuvant Temozolomide in patients with newly diagnosed, surgically resected, EGFRvIII-positive glioblastoma (ACT IV). Experimental: Rindopepimut /GM-CSF plus Temozolomide Active Comparator: KLH plus Temozolomide Each: Two intradermal injections two weeks apart, followed by monthly injections until tumor progression or intolerance.


Radiological Diagnosis
Neurosurgical Resection
Patient-specific
Peptide Vaccination
Combined Radio- and Chemotherapy
6 weeks: 30 x 2 Gy + 75 mg/m2/d Temozolomide
4 weeks break
Monitoring of response
Tissue collection
PBMCs
T cells
DNA
HLA typing
Whole genome sequencing
Tumor tissue
Glioblastoma cells
GSCs
HLA ligands
Whole genome sequencing
T cell specificity verification
Peptide synthesis and modification
Antigen selection
GMP-conform peptide synthesis
Ideal antigen:
• Multi-peptide vaccine
• Patient-specific / individual
• Glioma stem cell (GSC) targeting
• Good HLA binding properties
• Mutated antigens
• Promote CD8+ and CD4+ T cell response


Glioblastoma Tumor cell
cytotoxic
T cell
Immunological
synapse
CD8
CD3
TCR
peptide:HLA
class I complex
Proteasomic
cleavage
Proteasome
Protein carrying
mutation
Normal Peptide
Translation into
Proteins
Mutated Peptide
Membrane fusion
TAP
DNA Mutation
Peptide loading
onto HLA class I
Golgi apparatus
Nucleus
Endoplasmatic
reticulum


Heterogenous glioblastoma cell mass:
• cancer cells
• cancer initiating cells
• immune cells
• endothelial cells
glioblastoma tumor cell
CNS
PGE2
TGF-β
IL-10
TGF-β
IL-6
local APC
regulatory T cell
Perforin
Granzym
Granulysin
IL-10
IDO
TGF-β
IL-10
tumor-specific cytotoxic T cell
Reactivation
Blood Brain Barrier
IFN-γ
T helper 1 phenotype
IL-2
3) Migration
2) Proliferation / Clonal expansion
1) Priming / Preactivation
IL-2
Periphery
Cytokines (e.g. IL-7 or IL-21)
Multipeptide Vaccine
CD8+ cytotoxic T cell
CD4+ T helper cells
MHC class I
MHC class II
costimulatory molecules
+
Adjuvant (e.g. TLR-Agonists)
peripheral Dendritic Cell (DC)
Priming of peripheral anti-tumor response:
• antigen presenting Dendritic Cell
• CD4+ T cells (Th1 phenotype)
• CD8+ cytotoxic T cells
• boosting immune response
Imiquimod, IL-7 and IL-21